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Systemic Toxicity of Sulphur Mustard (HD) in Domestic Swine

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Canada

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Systemic Toxicity of Sulphur Mustard (HD) in Domestic Swine

W7702-9-R733/001/EDM

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Executive Summary

The systemic effects of different intravenous (iv) doses of sulphur mustard (HD) were evaluated in an anaesthetized domestic swine model (DSM).

Physiological, hematological, biochemical, and clinical parameters were evaluated and compared to anaesthetized controls.

There was a dose-dependent response to HD, which is demonstrated best in the physiological parameters monitored and collected under general anesthesia (GA).

Tissue was collected post-mortem for histopathological evaluation. Dr. P.N Nation of Veterinary Pathology Laboratory, 9520 27th Ave., Edmonton, AB conducted this examination

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Introduction

Sulphur mustard (HD) has been, perhaps, the most frequently deployed chemical warfare (CW) agent in modern history.

Research surrounding HD has emphasized cutaneous rather than systemic toxicity. Nonetheless, systemic intoxication should be an operational concern Despite the fact that there have been casualties treated in state-of- the-art European medical facilities, there is minimal documentation of the systemic pathophysiology.

The objective of this study was to comprehensively assess the dose-response of intravenous (iv) HD in a domestic swine model (DSM) during a general anaesthetic and following recovery from GA for a period of up to 72 hours post-GA

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Methods

Animals:

9-10 week-old Yorkshire-Landrace cross barrows (*Sus scrofa domestica*) were obtained from a local supplier and housed indoors at the vivarium complex at Defence Research Establishment Suffield (DRES) Animals received 16% hog grower and were allowed tap water *ad libitum*. The mean body weight at the time of surgery was 21.0 kg ± 2 07 (mean ± SD, n=19).

In conducting this research the authors adhered to the "Guide to the Care and Use of Experimental Animals" and "The Ethics of Animal Experimentation" published by the Canadian Council on Animal Care.

Anesthesia:

Animals were fed until the evening prior to surgery and allowed tap water *ad libitum* until the time of the experiment. The animals were not premedicated. After weighing, all animals underwent an inhalation induction in the transport container Isoflurane 5% (Abbott Laboratories, Montreal, Quebec, Canada) in oxygen (O_2) as a carrier gas at a flow rate of 8 liters per minute (L/min) was administered utilizing a Boyle anesthesia machine with a circle patient anesthesia circuit. Post-induction the animal was positioned in the dorsal recumbent position on a heated operating table The isoflurane concentration was reduced to 3% in 100% O_2 at a flow rate of 1 L/min. Animals were intubated with a 6.0 mm internal diameter (I.D.) cuffed oral endotracheal tube (Ruschelit, Willy Rusch AG, 71394 Kernen, Germany) and the cuff was inflated with room air to achieve a seal Central venous access was established by means of a cut-down into the right internal jugular vein. A 20 gauge iv catheter was inserted directly into the right carotid artery for blood pressure measurement and blood sampling. All catheters were fixed to the skin and the edges of the incision approximated.

Core Body temperature was maintained at approximately 38.5 °C by means of a Sage-London Industries Inc. Therm-o-matic heated operating table.

At the end of the experimental period, the artery and vein were doubly ligated, hemostasis was assured, the incisions were infiltrated with 0.5% bupivacaine (Marcaine, Sanofi Canada Markam, ON, Canada) diluted to 0.25% with sterile normal saline (sodium chloride 0.9%, Abbott Laboratories Ltd.), and all incisions closed in layers.

Aseptic technique was maintained at all times.

The animal was transported to a separate pen while intubated The recovery period was monitored and observations recorded

Concomitantly with the anaesthetic, all animals received iv normal saline (sodium chloride 0.9%, Abbott Laboratories Ltd.) at a rate of 9.00 ml/kg/hr ± 0.59 ml/kg/hr (mean ± SD, n=19) utilizing a volumetric infusion pump (Travenol FloGard 8000, Travenol laboratories). Urinary output was measured at 3 72 ml/kg/hr ± 1.45 (mean ± SD, n=19) via a percutaneously placed needle cystostomy.

Monitors:

A pulse oximeter to measure percentage oxygen saturation of arterial blood (SpO2) was connected to a multifunction monitor (Nellcor N1000, Nellcor Inc., Hayward, CA)) which was in turn connected via analog outputs to a Coulbourn Instruments high-speed videograph I/O port (L19-02, Coulbourn Instruments, Inc , Allentown, PA). Data was collected via a Coulbourn Instruments LabLinc S computer interface (Coulbourn Instruments, Inc , Allentown, PA) to an ALR 386 IBM compatible PC Data was displayed and stored using WinGraph for Windows software.

To measure blood pressure the catheter in the right carotid artery was connected to a transducer (Cobe CDXpress 3cc) which, in turn, was connected to a Coulbourn Instruments type A transducer coupler (S72-75). Data was collected via a Coulbourn Instruments LabLinc S computer interface (Coulbourn

Instruments, Inc., Allentown, PA) to an ALR 386 IBM compatible PC. Data was displayed and stored using WinGraph for Windows software.

Body temperature was recorded utilizing a rectal thermistor probe connected to a Coulbourn Instruments temperature S71-30 coupler. Core temperature was maintained at approximately 38.5 °C by means of a Sage-London Industries Inc. Therm-o-matic heated operating table. Data was collected via a Coulbourn Instruments LabLinc S computer interface to an ALR 386 IBM compatible PC. Data was displayed and stored using WinGraph for Windows software.

Airway pressure and flow were measured at the proximal end of the endotracheal tube utilizing a VarFlex flow transducer (Bear Medical Systems, Inc, Riverside, CA). Esophageal pressure was measured utilizing an 8FR SmartCath catheter (Bear Medical Systems, Inc, Riverside, CA). Proper positioning of the intraesophageal balloon was confirmed utilizing an occlusion test. These transducers were connected to a Bicore CP-100 adult pulmonary monitor (Bear Medical Systems, Inc, Riverside, CA). Data was collected via a serial output to an ALR FlexCache SX 386Z IBM compatible PC

Experimental Protocol.

Venepuncture for baseline "awake" samples for hematology and biochemistry, including total cholinesterase activity (ChE), was performed immediately prior to placement in the transport container. Animals were weighed in the transport container. After the monitors were established the animals were allowed a stabilization period of at least 30 min, during which time steady-state anesthesia was established Baseline physiological and biochemical values were obtained prior to drug administration and measurements were continued for the duration of anesthesia and displayed on a standard CRT monitor These measurements included temperature (°C), respiratory frequency (f, breaths/min), and SpO2 (%)

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Blood Samples:

Blood was collected for hematological and biochemical analysis at the following time points:

1-awake control samples taken prior to weighing

2-under steady-state GA immediately prior to the administration of HD or "sham" HD

3-under steady-state GA 1 hr post-HD or "sham" HD

4-under steady-state GA 3 hr post-HD or "sham" HD

5-under steady-state GA 5 hr post-HD or "sham" HD

6-24 hr post-HD or "sham" HD

7-48 hr post-HD or "sham" HD

8-72 hr post-HD or "sham" HD

The following biochemical parameters were examined:

TP	total protein
Alb	albumen
Glob	globulin
Chol	cholesterol
Trig	triglycerides
Glu	glucose
Ca^{++}	calcium
Mg^{++}	magnesium
Phos	phosphate
TBil	total bilirubin
AST	aspartate aminotransferase (SGOT)
ALT	alanine aminotransferase (SGPT)

GGT	gamma-glutamyltransferase
AlkP	alkaline phosphatase
LDH	lactate dehydrogenase
CK	creatine kinase
Amyl	amylase
Lipa	lipase
Crea	creatinine
Urea/BUN	urea/blood urea nitrogen
Uric	uric acid

The following electrolyte parameters were examined

K^+	potassium
Na^+	sodium
Cl^-	chloride

The following hematological parameters were examined:

Hct	haematocrit
Hgb	haemoglobin
MCHC	mean corpuscular haemoglobin concentration
WBC	white blood cell
Gran	absolute granulocytes
% Gran	% granulocytes
L/M	absolute lymphocytes/monocytes
% L/M	% lymphocytes/monocytes
Eos	eosinophils
PLT	platelets
Retics	reticulocytes

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Cholinesterase (ChE) Activity:

Blood was collected throughout the experimental period for total ChE determination by radiometric assay.

Blood samples for ChE determination were collected at the following time points:

1-awake control samples taken prior to weighing

2-under steady-state GA immediately prior to the administration of HD or "sham" HD

The inspired isoflurane concentration was reduced to 2% in 100% O_2 at a flow rate of 1 L/min After a 20 minute equilibration period three 0.5 ml samples of arterial blood for control total ChE were drawn at 5 minute intervals

3-under steady-state GA at 15 min (q 15 min) post-HD or "sham" HD for the duration of the anaesthetic

4-24 hr post-HD or "sham" HD

5-48 hr post-HD or "sham" HD

6-72 hr post-HD or "sham" HD

Euthanasia:

Animals considered to be suffering excessively or to be moribund were euthanised post-anesthesia with an iv injection of 10 ml (65mg/ml) sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge, ON, Canada) at the discretion of the scientific authority (SA) Otherwise, animals were euthanised 72 hr post-HD or post-"sham".

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Post-Mortem Examination:

Animals were weighed following euthanasia and transported to the autopsy suite in the vivarium complex at DRES. Tissues/samples were collected in accordance with a protocol established by Dr. N Nation. This protocol may be found on p.12. Samples were subsequently transferred by commercial carrier to his laboratory for examination.

Skin	
Salivary Gland	
Brachial Artery/Nerve	
Tongue	
Thyroid Glands	
Trachea	
Esophagus	
Heart	L Vent Free Wall
	R Vent Free Wall
	Intravent Septum (EM)
	Atrium
Aorta	
Lung	Caudal-Pleura
	-Bronchus
	-adjacent to Main Stem Bronchi
Lung	Anterior Lobe
Lung	Middle Lobe
Spleen	
Pancreas	
Duodenum	
Sigmoid Colon	
Spiral Colon	
Caecum	
Ileum	
Jejeunum	
Stomach	Glandular
	Bile Sample
Gall Bladder	
Liver	
Adrenals	
Kidney	
Inguinal Lymph Nodes	
	Urine Sample
Bladder	
Urethra	
Femoral Artery/Vein/Nerve	
Intercostal Muscle (EM)	
Quadriceps	
Femoral Bone Marrow	
Diaphragm	Edge
	Middle
	(EM)
Spinal Cord	Cervical
	Thoracic
	Lumbar
Brain	
Eyes	
Optic Nerves	
Pituitary	
Gasserian Ganglion (EM)	

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Results

Clinical:

HD TOXICITY-MASTER TABLE

HD DOSE (mg/kg)	CONTROL	?	1	3	5	7
#1	99-216[1,2]		99-228[1]	*99-232*	99-219[1]	99-225[1,4]
#2	99-222[1]		*00-241*	*99-235*	99-221[1,3]	
#3	99-227[1]		*00-243*	*99-236*	99-224[1,3]	
#4	*99-231*		*00-299*	*00-244*	*99-233*	
#5	*99-234*				*00-245*	
#6	*00-240*				*00-250*	
#7	*00-247*					
#8	*00-248*					
#9	*00-249*					
#10	*00-278*[5]					
#11	*00-279*					
#12	*00-298*					

Animals included in Analysis

1- 2 general anesthetics
2- small iv infusion
3- intra-op death
4- euthanised on table
5- ?infection

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INTRA-OPERATIVE FLUID BALANCE:

Control:

Fluids	#1[1] (231)	#2 (234)	#3 (240)	#4 (247)	#5 (248)	#6 (249)	#7[5] (278)	#8 (279)	#9 (298)					Avg	Avg*
intake	8 59	8 77	7 88	9.33	9 16	9 68	10 21	9 25	8 38					9 03	8 92
output	3 93	4 28	3 12	4 50	4 08	7 08	2 88	5 34	3 22					4 27	4 52

1 ?hypoxic injury
5 ?infection
* #231 and #278 ***NOT*** included

HD 1 mg/kg:

Fluids	#1 (228)	#2 (241)	#3 (243)	#4 (299	#5	#6	Average*
intake	7.90	9.67	9.07	8.14			8.96
output	1.52	5.70	3.71	3.57			4.32

2 #228 ***NOT*** included

HD 3 mg/kg:

Fluids	#1 (232)	#2 (235)	#3 (236)	#4 (244)	#5	#6	Average
intake	8 68	8.60	8.63	9.43			8.84
output	2.16	4.01	0.76	3.89			2 71

HD 5 mg/kg:

Fluids	#1 (233)	#2 (245)	#3 (250)	#4	#5	#6	Average
intake	8.65	9.48	9 31				9 15
output	2.03	2.05	4 40				2 83

Summary of Intra-operative Fluid Balance:

Fluids	control	1 mg/kg	3 mg/kg	5 mg/kg
intake	8.92	9.19	8.84	9.15
output	4.52	4.50	2.71	2.83

These data are summarized in the Figure 1.

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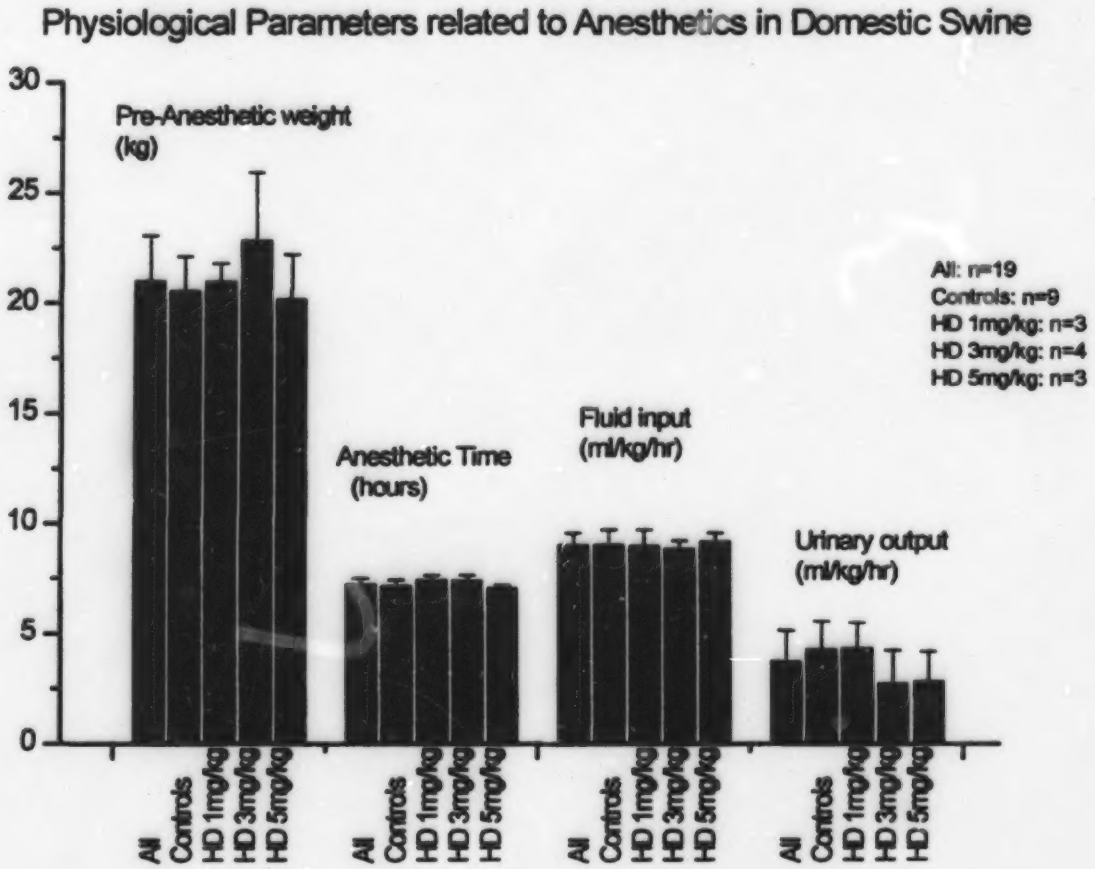
FIGURE 1



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Intra-operative physiological parameters in each group are summarized as follows:

Figure 2	mean arterial blood pressure
Figure 3	oxygen saturation
Figure 4	temperature
Figure 5	inspirational tidal volume, expirational tidal volume, frequency, minute ventilation
Figure 6	peak inspirational flow rate, expiratory airway resistance, peak expirational flow rate, mean airway resistance
Figure 7	work of breathing, dynamic compliance, mouth occlusion pressure, fraction of inspirational time/breath

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FIGURE 2

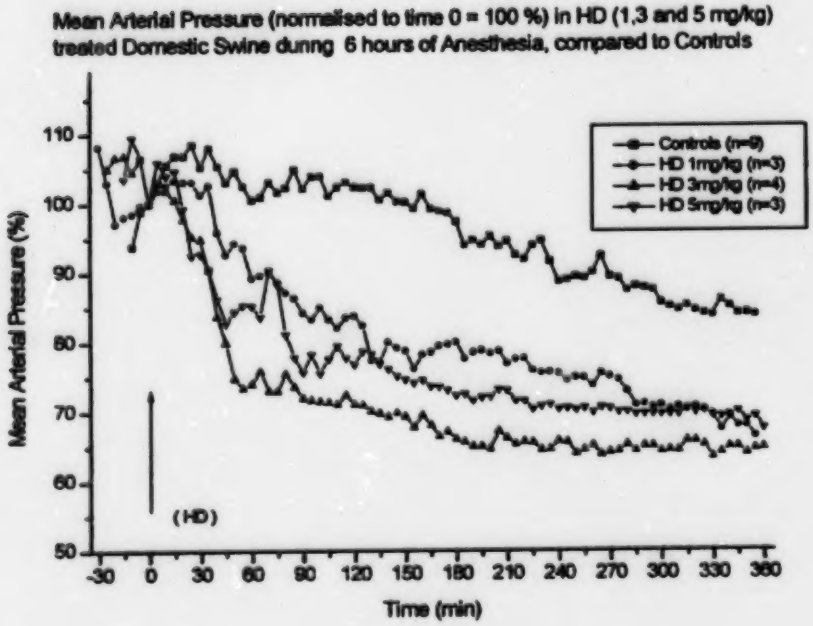

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FIGURE 3

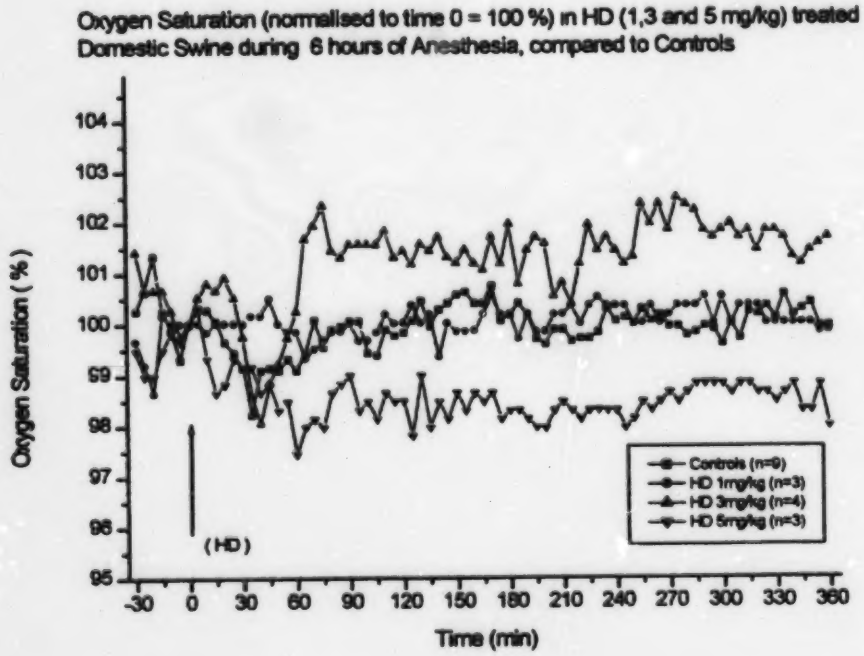

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FIGURE 4

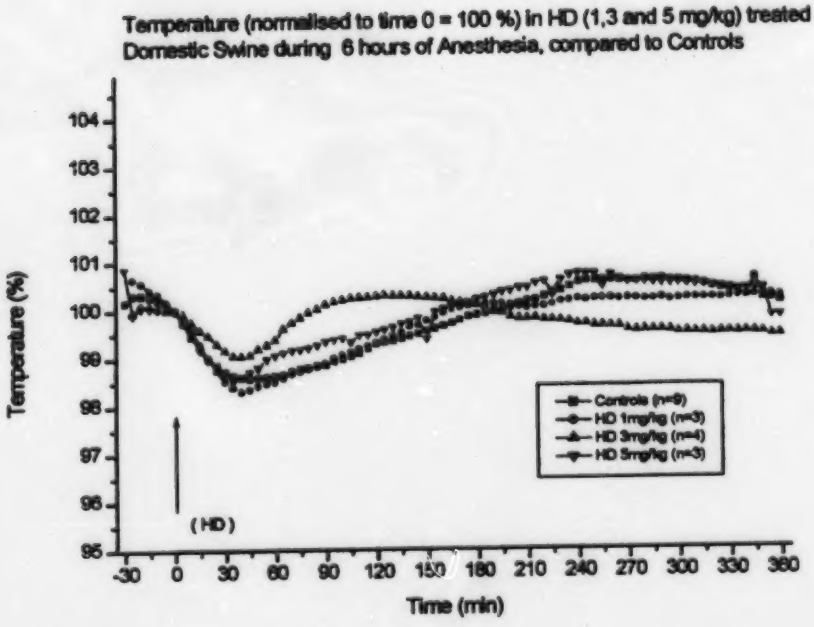

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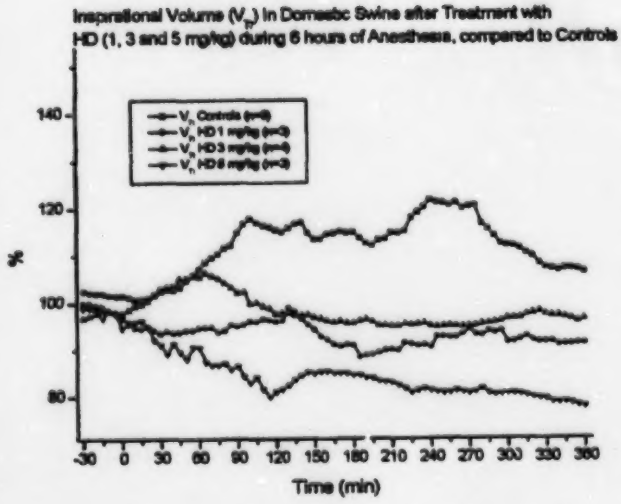
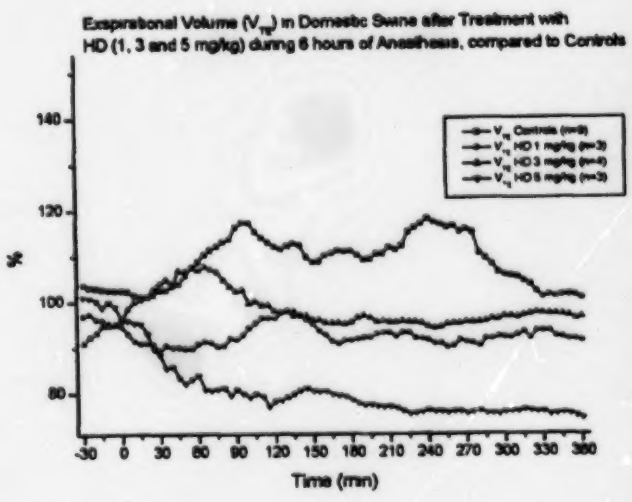
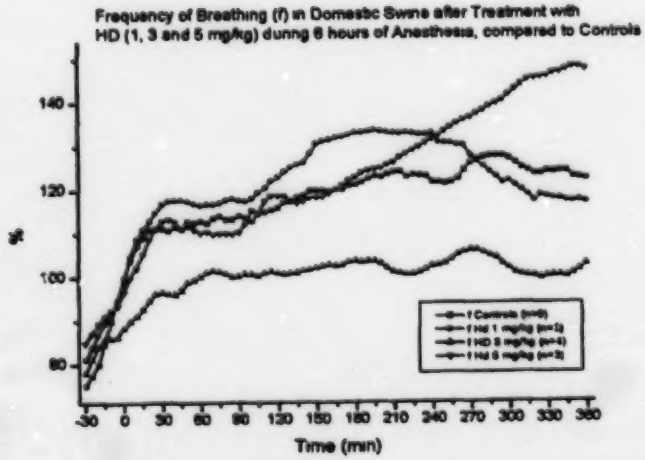
FIGURE 5


Inspirational Volume (V_{Ti}) in Domestic Swine after Treatment with HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
%
Time (min)


Expirational Volume (V_{Te}) in Domestic Swine after Treatment with HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
%
Time (min)


Frequency of Breathing (f) in Domestic Swine after Treatment with HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
%
Time (min)

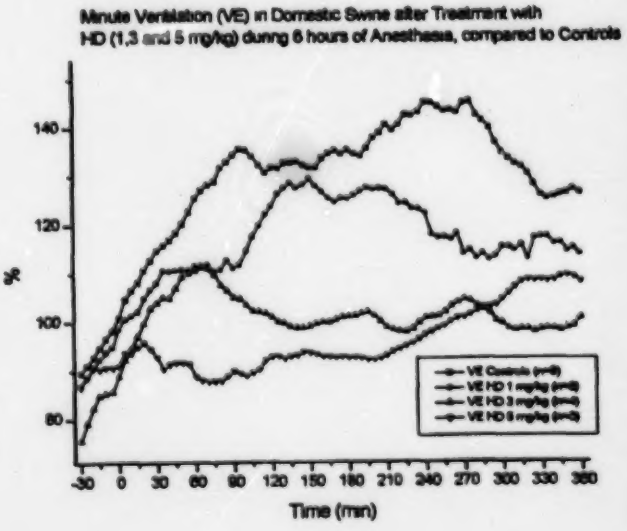

Minute Ventilation (VE) in Domestic Swine after Treatment with HD (1,3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
%
Time (min)

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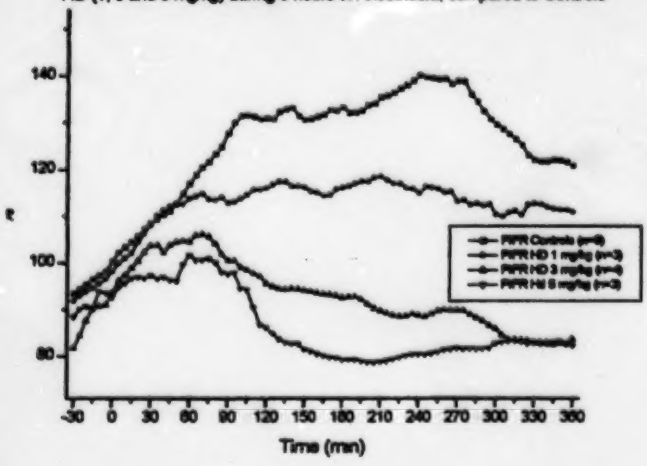
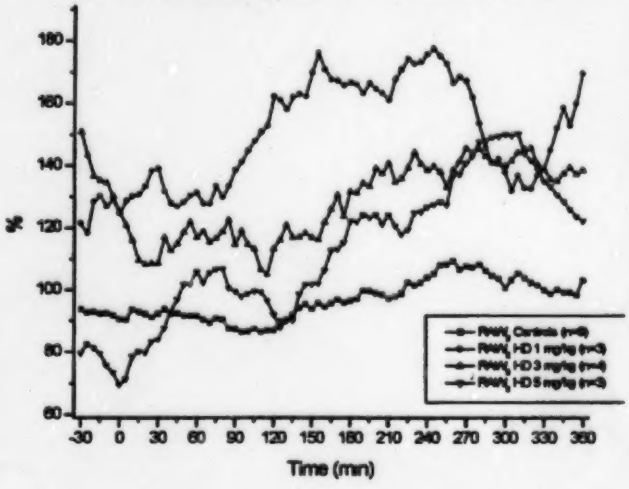
FIGURE 6

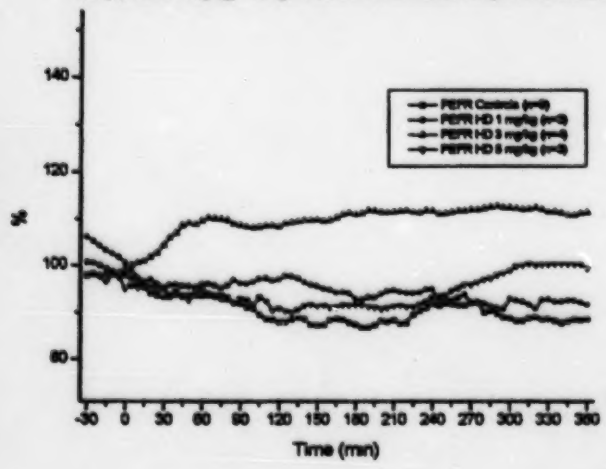
Peak Inspiratonal Flow Rate (PIFR) in Domestic Swine after Treatment with HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls



Expiratory Airway Resistance (RAW_e) in Domestic Swine after Treatment with HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls



Peak Expiratonal Flow Rate (PEFR) in Domestic Swine after Treatment with HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls



Mean Airway Resistance (RAW_m) in Domestic Swine after Treatment with HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls

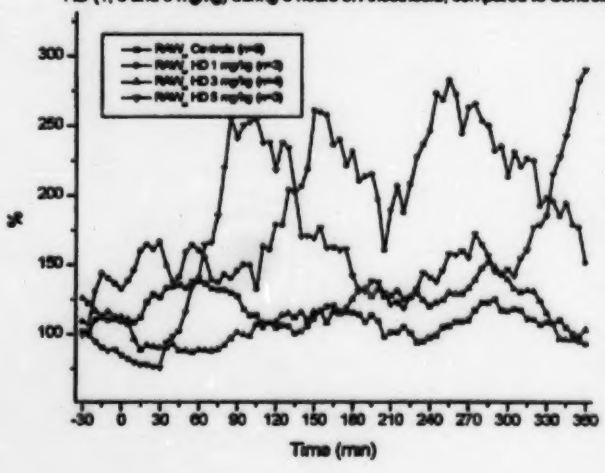


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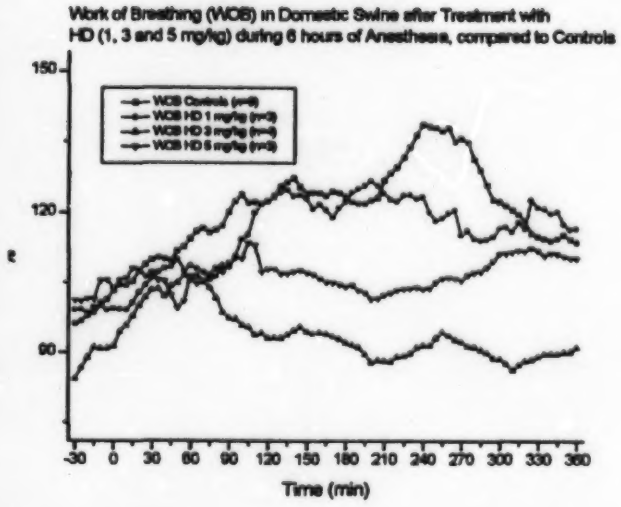
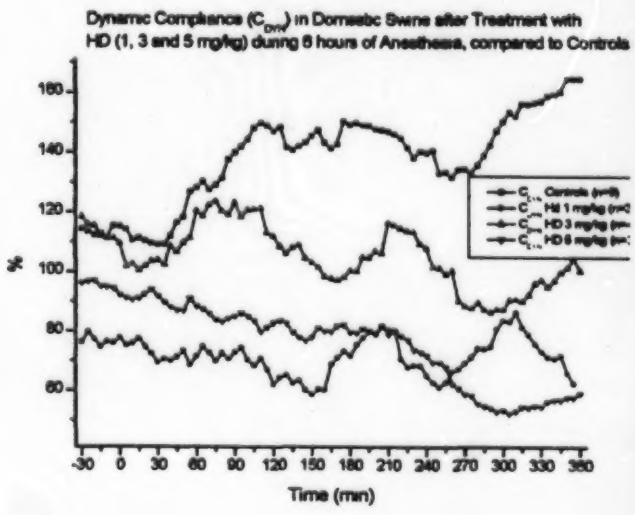
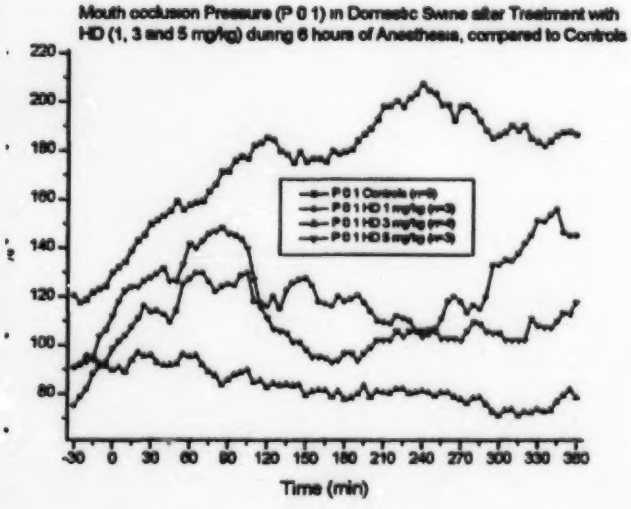
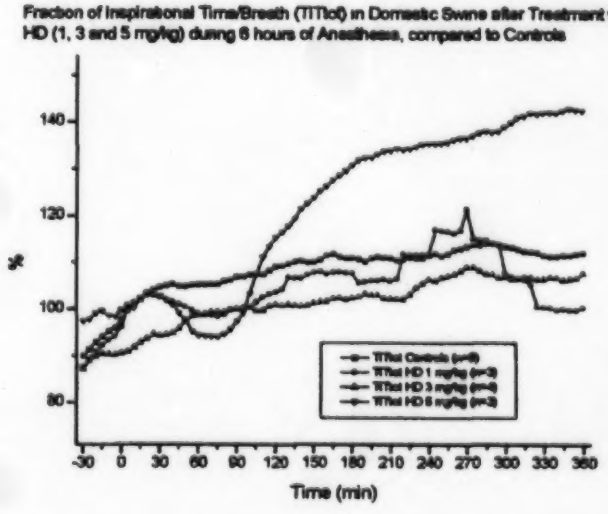
FIGURE 7


Work of Breathing (WOB) in Domestic Swine after Treatment with
HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
150
120
90
-30 0 30 60 90 120 150 180 210 240 270 300 330 360
Time (min)


Dynamic Compliance (C_{Dyn}) in Domestic Swine after Treatment with
HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
160
140
120
100
80
60
-30 0 30 60 90 120 150 180 210 240 270 300 330 360
Time (min)


Mouth occlusion Pressure (P 0.1) in Domestic Swine after Treatment with
HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
220
200
180
160
140
120
100
80
-30 0 30 60 90 120 150 180 210 240 270 300 330 360
Time (min)


Fraction of Inspiratonal Time/Breath (TI/Tot) in Domestic Swine after Treatment with
HD (1, 3 and 5 mg/kg) during 6 hours of Anesthesia, compared to Controls
140
120
100
80
-30 0 30 60 90 120 150 180 210 240 270 300 330 360
Time (min)

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RECOVERY PERIOD

Control:

Signs	#1[1] (231)	#2 (234)	#3 (240)	#4 (247)	#5 (248)	#6 (249)	#7[5] (278)	#8 (279)	#9 (298)					Avg[*]
Extubation	13	9	15	12	8	10	17	10	8					10 3
Righting		10	17	12	8	11	22	11	12					11 6
Stand (a)	21	10		20	19	(19)	25		15					16 6
Stand (b)	53	29	73	>60	20	19	27	13	16					28 3
Cough	9			17		10								43 (%)
Vomit	?27	?18	101											43 (%)
Urine	26		17	2	2									57 1 (%)
Defec	33													14 3 (%)
Eat														
Drink														
Other														

1 ?hypoxic injury
5 ?infection
Stand (a)-attempting to stand
Stand (b)-standing
* #231 and #278 ***NOT*** included

HD 1 mg/kg:

Signs	#1 (228)	#2 (241)	#3 (243)	#4 (299)	#5	#6	Average*
Extubation	6	14	9	22			15.0
Righting	10	8	10	28			15.3
Stand (a)	23	56	16	29			33 7
Stand (b)	23	58	21	33			37.3
Cough		8					8 33%
Vomit							0%
Urinate	13	22	14				16.3 100%
Defecate							0%
Eat			24				24 33%
Drink							
Other							

Stand (a)-attempting to stand
Stand (b)-standing
* #228 ***NOT*** included

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HD 3 mg/kg:

Signs	#1 (235)	#2 (236)	#3 (244)	#4	#5	#6	Average
Extubation	6	8	17				10.3
Righting	9	27	17				17.7
Stand (a)	25	64	>60				49.7+
Stand (b)							
Cough	6	7					66%
Vomit	18	34					26.0 66%
Urinate	43		7				25.0 66%
Defecate		14					14.0 33%
Eat							
Drink							
Other	Neurol.	?neurol	emesis				+++ 100%

Stand (a)-attempting to stand
Stand (b)-standing

HD 5 mg/kg:

Signs	#1 (233)	#2 (245)	#3 (250)	#4	#5	#6	Average
Extubation	5	27	7				13.0
Righting	4	?	13				?9+
Stand (a)	10	>60	15				35+
Stand (b)	?26		?				>28
Cough	-	-	-				-
Vomit	20	42	14				25.0 100%
Urinate	22	25	-				23.5 66%
Defecate	20	30	12				10.8 100%
Eat							
Drink							
Other			NM				33%

Stand (a)-attempting to stand
Stand (b)-standing

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Summary of Recovery Period:

Signs	Control	1 mg/kg	3 mg/kg	5 mg/kg			
Extubation	10 3	15.0	10.3	13.0			
Righting	11 6	15.3	17 7	?9+			
Stand (a)	16 6	33.7	49.7+	35+			
Stand (b)	28 3	37.3		>28			
Cough	43%	8 33%	66%	-			
Vomit	43%	0%	26.0 66%	25.0 100%			
Urinate	57.1%	16.3 100%	25.0 66%	23.5 66%			
Defecate	14.3%	0%	14.0 33%	10.8 100%			
Eat		24 33%					
Drink							
Other			+++ 100%	33%			

Stand (a)-attempting to stand
Stand (b)-standing

Post-anaesthetic/recovery parameters are summarized in Figure 8

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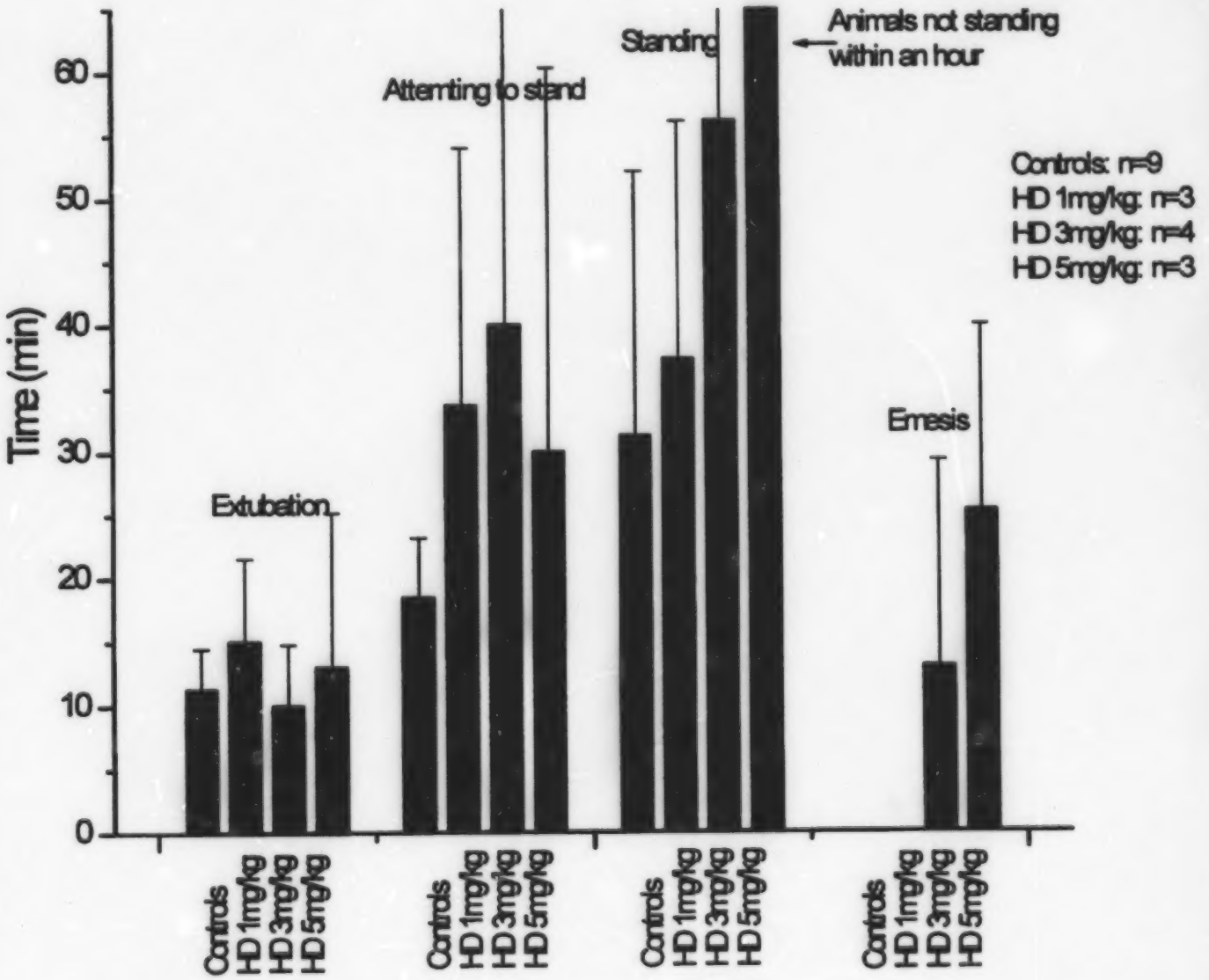
FIGURE 8

Post-Anesthetic observations (Average Time ± SD) in Animals treated with HD (1,3 and 5 mg/kg), compared to Controls



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Pig #	Group	Pre-Anaesthetic	Post-Mortem	% Change	Group Avg.
231	control	17.7	18.8	(1 1) + 6.21	*+ 2.74%*
234	"	20.7	21.2	(0 5) + 2.42	
240	"	20.3	21 4	(1.1) + 5.42	
247	"	23.7	24.6	(0 9) + 3.80	
248	"	20.9	21 0	(0.1) + 0.48	
249	"	20.4	20.6	(0.3) – 1 44	
279	"	21.3	----------		
298	"	19.3	----------		
241	1 mg/kg	20.3	19.4	(0 9) – 4.43	*- 2.38%*
243	"	20.7	22.0	(1.3) + 6.28	
299	"	21.9	20.0	(1 9) – 8.68	
232	3 mg/kg	19.5	16.2	(3.3) –16.92	*- 12.17%*
235	"	21.3	18.9	(2.4) –11 27	
236	"	26.7	22.4	(4 3) –16.10	
244	"	23.7	22.6	(1.1) – 4 64	
233	5 mg/kg	17.7	14.7	(3 0) –17.65	*- 13.27%*
245	"	21.3	19.2	(2.1) – 9 86	
250	"	21.3	18.4	(2.9) –13.62	

Pre-anaesthetic and post-anaesthetic weights are summarized in Figure 9

Systemic Toxicity of Sulphur Mustard in Domestic Swine

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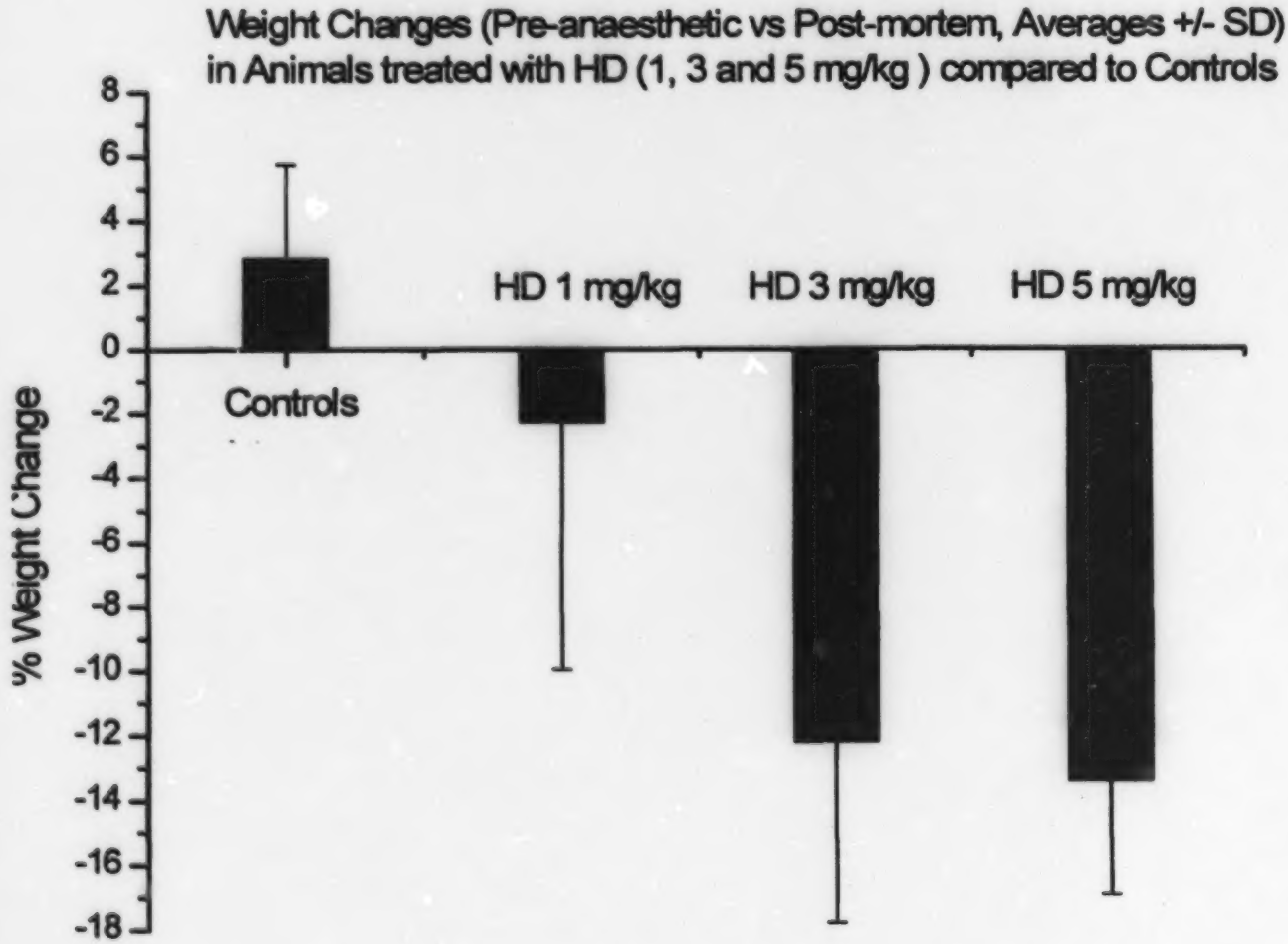
FIGURE 9



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Hematological, biochemical, and electrolyte values for control and HD treatment groups are summarized as follows:

Table 1	awake values
Table 2	1 hour post-treatment
Table 3	3 hours post-treatment
Table 4	5 hours post-treatment
Table 5	24 hours post-treatment
Table 6	24 and 72 hours post-treatment
Figure 10	total cholinesterase activity

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Table 1: Awake Values, HD 1, 3 and 5 mg, compared to Controls

	Controls	HD 1 mg	HD 3 mg	HD 5 mg
TP g/l	57	57	58	59
ALB g/l	30	29	31	30
Ca mmol/l	2.65	2.65	2.71	2.71
Mg mmol/l	0.96	1.02	0.94	0.87
Phos mmol/l	3.04	3.09	2.92	2.90
AST u/l	34	34	52	46
GGT u/l	50	51	41	44
ALKP u/l	266	266	275	255
TBIL umol/l	5	3	4	3
LDH u/l	1956	2021	2009	1708
CK u/l	446	229	392	237
CHOL mmol/l	3.01	2.99	2.61	2.80
TRIG mmol/l	0.48	0.42	0.55	0 48
GLU mmol/l	6.06	5.72	6.99	5.45
AMYL u/l	398	454	429	355
LIPA u/l	10	21	33	13
CREA u/l	100	107	105	104
Urea/BUN mmol/l	4.04	3.32	3.96	3.94
URIC umol/l	11.11	9.67	12.00	9 67
Na mmlo/l	148.50	148.77	147.90	146.67
K mmol/l	6.03	5.06	5.22	4 73
Cl mmol/l	107	105	106	105

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Table 1: Awake Values, HD 1, 3 and 5 mg, compared to Controls-continued

HCT %	36.09	39.57	35.75	36 10
HGB g/dl	12.10	13.27	12 28	12.10
MCHC g/dl	33.54	33.53	34 50	33.50
WBC 10^9/L	14.48	19.33	18.08	15.93
GRAN 10^9/L	6.92	10.33	10.00	7.47
% GRAN	47.56	52.67	55.50	47.00
NEUT 10^9/L	6.90	7 05	9 80	6 10
EOS 10^9/L	1.45	3.85	1.90	1.80
L/M 10^9/L	7.52	9.00	8.08	8.47
% L/M	52.44	47.33	44.50	53.00
PLT 10^9/L	521.22	340.67	507.25	560.67
RETICS %	1.10	4.00	0.25	

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Table 2: Values 1 h after Agent, HD 1, 3 and 5 mg, compared to Controls (C: animals anesthetized, no agent).

	Controls	HD 1mg	HD 3 mg	HD 5 mg
	n=9	n=3	n=4	n=3
TP g/l	47	46	46	48
ALB g/l	23	22	23	24
Ca mmol/l	2.50	2.39	2.59	2.64
Mg mmol/l	0 83	0.77	0 94	0.85
PHOS mmol/l	2.75	2.80	2.55	2.61
AST u/l	30	41	15	9
GGT u/l	38	39	33	35
ALKP u/l	224	212	219	199
TBIL umol/l	2	1	3	2
LDH u/l	1639	1892	1678	1445
CK u/l	240	309	365	190
CHOL mmol/l	1.93	1.94	1.76	2.08
TRIG mmol/l	0.18	0.16	0.18	0.16
GLU mmol/l	5.92	6.99	7.26	6.54
AMYL u/l	316	381	334	265
LIPA u/l	0	9	0	0
CREA u/l	92	92	92	100
Urea/BUN mmol/l	3.33	3.02	4.98	3 88
URIC umol/l	11	11	11	11
Na mmol/l	145	144	143	145
K mmol/l	4.25	4.04	4.17	3.93
Cl mmol/l	106	106	105	107
HCT %	26.9	27.9	24.8	26.3
HGB g/dl	9.5	9.9	9.1	9.2
MCHC g/dl	35.4	35.5	36.5	35.0
WBC 10^9/L	13.5	12.8	10.7	18.2
GRAN 10^9/L	9.5	8.4	7.6	14.0
% GRAN	70	66	72	75
NEUT 10^9/L	8.7	–	7.0	12 2
EOS 10^9/L	1.2	–	1.1	1.8
L/M 10^9/L	4.0	4.4	3.1	4.2
% L/M	30	34	29	25
PLT 10^9/L	290	342	344	340
RETICS %	0.8			

Systemic Toxicity of Sulphur Mustard in Domestic Swine

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Table 3: Values 3 h after Agent, HD 1, 3 and 5 mg, compared to Controls (C.: animals anesthetized, no Agent)

	Controls	HD 1 mg	HD 3 mg	HD 5 mg
	n=9	n=3	n=4	n=3
TP g/l	47	47	46	46
ALB g/l	23	23	22	23
Ca mmol/l	2.57	2.43	2.53	2.38
Mg mmol/l	0.84	0.80	0.95	0 87
PHOS mmol/l	2.76	2.96	2.84	2.96
AST u/l	24	18	23	22
GGT u/l	39	37	32	38
ALKP u/l	214	198	199	190
TBIL umol/l	2	0	3	2
LDH u/l	1624	1889	1527	1455
CK u/l	253	238	335	180
CHOL mmol/l	1 93	2.03	1.69	1.98
TRIG mmol/l	0.15	0.17	0.14	0 16
GLU mmol/l	6.95	7.35	7.63	6.57
AMYL u/l	303	368	318	256
LIPA u/l	0	[redacted]	0	0
CREA u/l	90	96	97	98
Urea/BUN mmol/l	3.37	3.11	4 70	3.85
URIC umol/l	12	11	13	13
Na mmlo/l	144	145	142	145
K mmol/l	4.49	4.33	4 49	4.68
Cl mmol/l	106	105	108	107

• values differ from Controls, $p < 0.05$, [redacted]

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Table 3: Values 3 h after Agent, HD 1, 3 and 5 mg, compared to Controls (C.: animals anesthetized, no Agent).

HCT %	25.7	28.2	25.9	26.2
HGB g/dl	9.1	10.0	8.9	9.2
MCHC g/dl	35.4	35.4	34.3	35.0
WBC 10^9/L	14.7	18.8	15.5	15.8
GRAN 10^9/L	11.3	14.8	13 0	13.0
% GRAN	77	78	84	79
NEUT 10^9/L	11.2	13.6	11.8	11.7
EOS 10^9/L	1.1	1.1	1 2	1.3
L/M 10^9/L	3.3	4.0	2.5	2.8
% L/M	23	22	17	21
PLT 10^9/L	255	312	373	391
RETICS %	0.3			0.5

* values differ from Controls, $p < 0.05$, [redacted]

Systemic Toxicity of Sulphur Mustard in Domestic Swine

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Table 4: Values 5 h after Agent, HD 1,3 and 5 mg, compared to Controls (C : animals anesthetized, no agent).

	Controls	HD 1 mg	HD 3 mg	HD 5 mg
	n=9	n=3	n=4	n=3
TP g/l	46	45	44	44
ALB g/l	22	21	21	22
Ca mmol/l	2.48	2 34	2 47	2.37
Mg mmol/l	0.76	0.74	0.91	0.90
PHOS mmol/l	2.72	[redacted]	[redacted]	[redacted]
AST u/l	25	17	18	17
GGT u/l	38	34	29	32
ALKP u/l	179	173	183	173
TBIL umol/l	2	2	3	2
LDH u/l	1607	1675	1523	1450
CK u/l	352	221	441	196
CHOL mmol/l	1.85	1.95	1.53*	1.79
TRIG mmol/l	0.07	0.13	0.14	0.09
GLU mmol/l	7.19	7.27	6.79	5.71
AMYL u/l	291	341	307	247
LIPA u/l	0	6*	2	2
CREA u/l	87	98	109*	105*
Urea/BUN mmol/l	3.54	3.27	5 22	4.69
URIC umol/l	12	13	[redacted]	14*
Na mmlo/l	143	146	143	144
K mmol/l	4.52	4.62	[redacted]	5.86*
Cl mmol/l	106	107	107	109*

* values differ from Controls, p=< 0.05 , [redacted]

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Table 4: Values 5 h after Agent, HD 1,3 and 5 mg, compared to Controls (C.· animals anesthetized, no agent).

HCT %	27.4	27.7	23.9	27.5
HGB g/dl	9.7	10.0	8.6	9.7
MCHC g/dl	35.6	35.9	36.1	35.1
WBC 10^9/L	14.8	15.1	13.5	9.4
GRAN 10^9/L	12 5	12.2	11.8	7.9
% GRAN	84	81	86	81
NEUT 10^9/L	12.7	10.4	13.0	8.7
EOS 10^9/L	1.0	0.9	1.1	1.0
L/M 10^9/L	2.3	2.9	1.6	1.5
% L/M	16	19	14	19
PLT 10^9/L	257	290	327	342
RETICS %	0.4		1.2	0.6

* values differ from Controls, p=< 0.05 , [redacted]

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Table 5: Values 24 h after anesthesia, HD 1, 3 and 5 mg, compared to Controls

	Controls	HD 1 mg	HD 3 mg	HD 5 mg
	n=9	n=3	n=4	n=2
				(No statistical analysis)
TP g/l	56	53	52	51
ALB g/l	27	27	27	28
Ca mmol/l	2.52	2.45	2.33	2.41
Mg mmol/l	0.85	0.87	0.96	0.91
PHOS mmol/l	2.84	3.01	3.14	3.98
AST u/l	231	155	691	342
GGT u/l	50	43	34	32
ALKP u/l	248	215	272	324
TBIL umol/l	2	1	4	5
LDH u/l	3060	4247	4739*	3216
CK u/l	6243	5904	3828	2375
CHOL mmol/l	2.12	2.90	2.56	2.85
TRIG mmol/l	0.65	0.80	0.41*	0.55
GLU mmol/l	6.58	7.68	6.75	9.93
AMYL u/l	360	369	389	357
LIPA u/l	48	23	106	128
CREA u/l	93	97	107	106
Urea/BUN mmol/l	5.26	4.70	[redacted]	11.55
URIC umol/l	11	11	13*	14
Na mol/l	151	154	150	150
K mmol/l	5.65	5.12	5.60	5.84
Cl mmol/l	107	109	108	111

*** values differ from Controls, $p < 0.05$, [redacted]**

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Table 5: Values 24 h after anesthesia, HD 1, 3 and 5 mg, compared to Controls

HCT %	32.7	41.4	34.6	47.7
HGB g/dl	11.1	13.5	12.0	16.3
MCHC g/dl	33.1	33.3	34.7	33.5
WBC 10^9/L	18.4	19.4	16.6	14.3
GRAN 10^9/L	10.9	14.2	15.8	8.4
% GRAN	57	74*	[redacted]	64
NEUT 10^9/L	13.4	14.0	2.6	2 8
EOS 10^9/L	1.2	0.9	5.9	3.7
L/M 10^9/L	7.5	5.2	[redacted]	5.9
% L/M	43	26*	[redacted]	37
PLT 10^9/L	367	392	426	257
RETICS %	0.4			

* values differ from Controls, $p < 0.05$, [redacted]

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Table 6: Values 48 and 72 h after anesthesia, HD 1 mg, compared to Controls

	Controls	HD 1 mg	Controls	HD 1 mg
	n=9	n=3	n=9	n=2
	48 h	48 h	72 h	72 h (no statistical analysis)
TP g/l	57	56	57	55
ALB g/l	28	27	28	28
Ca mmol/l	2.63	2.54	2.66	2.58
Mg mmol/l	0.95	0.84	0.92	1.04
PHOS mmol/l	2.83	2.70	2.84	2.94
AST u/l	69	72	47	55
GGT u/l	47	39	44	37
ALKP u/l	186	152	170	137
TBIL umol/l	5	3	4	5
LDH u/l	2466	2549	1996	2502
CK u/l	1350	1570	714	788
CHOL mmol/l	3.01	3.35	3.49	3.36
TRIG mmol/l	0.43	0 51	0.63	0.76
GLU mmol/l	5.96	6.44	5.95	7.95
AMYL u/l	320	352	324	317
LIPA u/l	14	15	11	8
CREA u/l	87	95	92	106
Urea/BUN mmol/l	3.41	3.04	3.89	3.77
URIC umol/l	11	10	11	11
Na mmlo/l	147	152	148	158
K mmol/l	6.58	6.18	6.21	4.85
Cl mmol/l	106	109	105	111

*** values differ from Controls, $p < 0.05$**

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Table 6: Values 48 and 72 h after anesthesia, HD 1 mg, compared to Controls

	Controls	HD 1 mg	Controls	HD 1 mg
	n=9	n=3	n=9	n=2
	48 h	48 h	72 h	72 h (no statistical analysis)
HCT %	32.7	39.4	32.6	41.1
HGB g/dl	11.0	13.5*	11.0	13 5
MCHC g/dl	34.0	34.3	33.8	33.0
WBC 10^9/L	16.9	18.8	18.5	10.9
GRAN 10^9/L	15.1	14.1	11.9	7.0
% GRAN	54	71	65	67
NEUT 10^9/L	14.0	23.6	8.8	4.5
EOS 10^9/L	1.9	1.4	4.3	2 5
L/M 10^9/L	7.2	4.7	6.6	4.0
% L/M	41	29	35	34
PLT 10^9/L	386	255	484	418
RETICS %	0.6	1.3		

* values differ from Controls, $p < 0.05$

Total cholinesterase activity in control and HD groups is summarized in figure 10.

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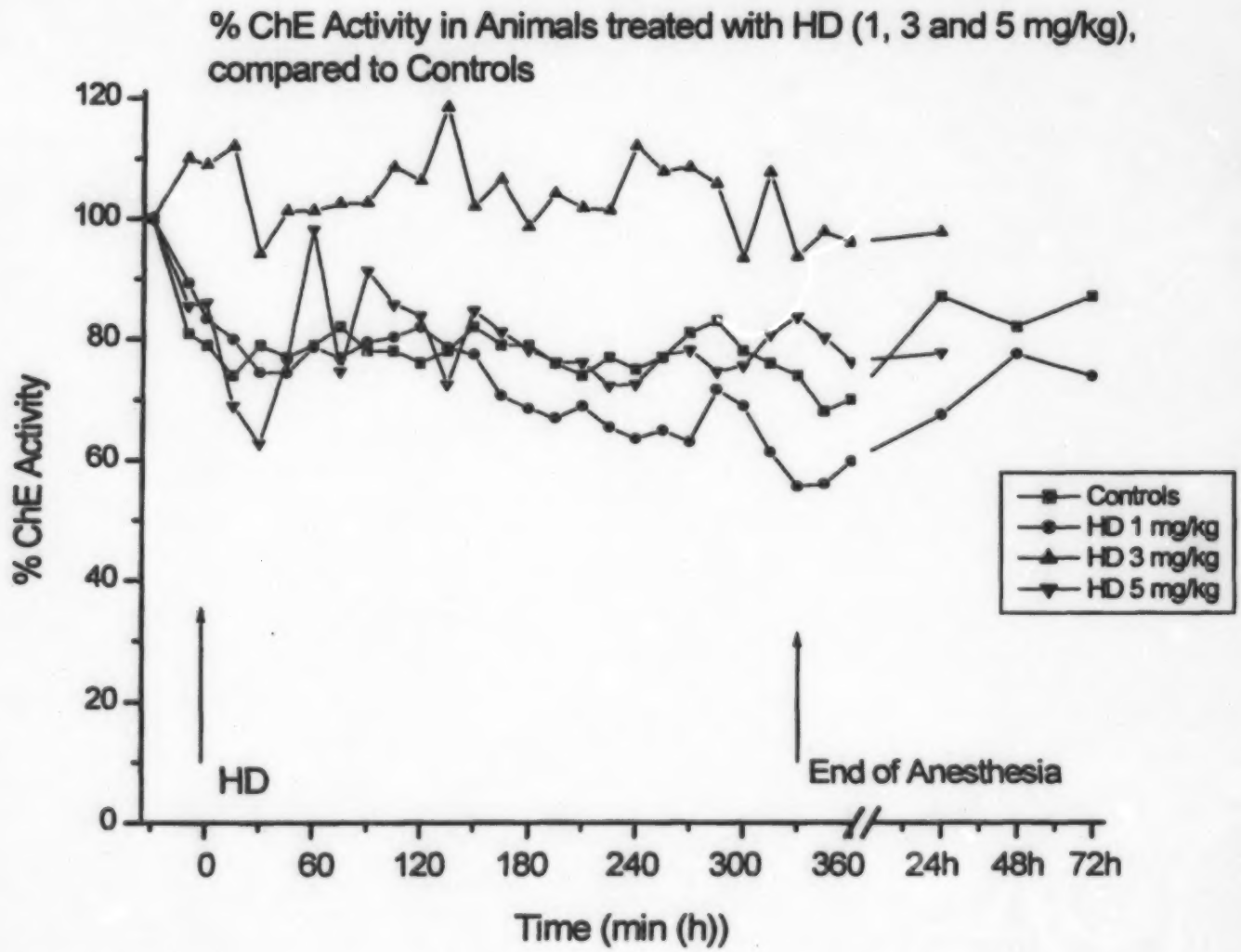
Figure 10



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Conclusions

There was no statistically significant difference between the control and HD treated groups with respect to pre-anesthetic weight, duration of anesthesia, fluid intake, and urinary output (Figure 1). However, the urinary output in the groups receiving 3 mg/kg and 5 mg/kg is clinically significant. This may be reflective of pre-renal, renal, or post-renal pathology.

Mean arterial pressure is significantly reduced in all HD treated groups relative to anesthetized controls (Figure 2). This may reflect primary cardiovascular pathology involving either the heart, the vasculature or both. Alternatively, the decline in mean arterial pressure may be secondary to central or peripheral effects of HD. Clearly, direct measurement of cardiac filling pressures, cardiac output and peripheral resistance with a flow-directed thermodilution cardiac output catheter would aid in understanding the pathophysiologic processes involved. This decline in pressure may explain the lower measured urinary output at higher doses of HD.

Oxygen saturation (Figure 3) is lessened only in the group receiving 5 mg/kg HD. This probably represents a dose-response. Because of the sigmoid shape of the oxyhemoglobin dissociation curve, oxygen saturation, as measured by pulse oximetry, does not always accurately reflect the oxygen tension in the blood. Furthermore, inferences regarding oxygen transport (cardiac output x oxygen content) and acid-base status cannot be made from this measurement.

Core temperature (Figure 4) is similar in all groups.

There appears to be a clear dose-response phenomenon occurring when one considers respiratory parameters (Figure 5) affecting minute ventilation (respiratory frequency x tidal volume). As expected, the relationship between inspiratory and expiratory tidal volume is maintained within each group. There appears to be a compensatory response for the decrease in tidal volume by increasing respiratory frequency, most evident at the highest dose of HD. It should be noted that this group also showed a decline in oxygen saturation. The rise in respiratory rate may be a direct or indirect reflex response to this decrease.

Measurement of peak inspiratory flow rate demonstrates a dose-related decrease in the HD treated groups compared to controls (Figure 6). While peak expiratory flows are similar in all groups, airway resistance is higher in the HD treated groups (figure 6)

Mouth occlusion pressure is a reflection of central respiratory drive (CRD) and the adequacy of respiratory musculature. Mouth occlusion pressure is decreased in all HD treated groups (Figure 7). The normal reciprocal relationship between resistance and compliance is seen (Figure 7). It is difficult to assess the inverse effect of respiratory frequency on compliance although these appear to correlate in a dose-related manner. Certainly, the direct correlation of respiratory frequency on inspiratory time holds.

The work of breathing is similar in all groups.

In summary, there appears to be a dose-related depression of minute ventilation, possibly as a result of decreased CRD This may be secondary to alterations in oxygenation or acid-base status. There is decreased dynamic compliance in the face of increase airway resistance. Animals receiving 1 mg/kg and 3 mg/kg decrease their respiratory rate which might be expected in the case of increased resistance work but do not increase tidal volumes

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Recovery from GA becomes increasingly complicated in a dose-related manner when HD treated groups are compared to controls Times to return of postural control are increased in a dose-related manner as are the incidence of gastrointestinal (GI) and neurological signs (Figure 8). Furthermore, HD treated animals exhibit ongoing GI signs (emesis, diarrhea) and disinterest in feeding. This is reflected in a dose-relate weight loss, whereas control animals gain weight post-operatively (Figure 9)

Hematological, biochemical, and electrolyte parameters examined pre-induction and 1 hour post-exposure are not significantly different in control or HD treated animals (Tables 1&2).

Lipase values are significantly elevated at 3 and 5 hours post exposure but only in the HD 1 mg/kg group (Tables 3&4). Additionally, phosphate is elevated in all 3 HD treated groups at 5 hours. Creatinine, uric acid and potassium are elevated at 5 hours in the 3 mg/kg and 5 mg/kg groups. It is of interest that these animals also had decreased urinary output.

At 24 hours post-GA (Table 5) the BUN differs significantly in the 3 mg/kg group. There are insufficient survivors in the 5 mg/kg group to demonstrate statistical significance, but BUN values suggest a dose related effect It should be noted that the elevation in BUN probably reflects pre-renal factors related to dehydration (emesis, diarrhea). The percentage of granulocytes increases and the number and percentage of lymphocytes/monocytes decreases.

At 48 hours (Table 6) only controls and 1 mg/kg animals survive and at 72 hours there are insufficient survivors in the HD treated group to analyze statistically.

Cholinesterase activity appears similar in all groups (Table 7). Note is made the return of activity to awake values in the 72 hours post-GA.

In summary, there do not appear to be any hematological or biochemical markers for HD intoxication. Changes seen appear secondary to surgery or clinical manifestations of systemic toxicity.

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